

What is claimed is:

1. A kit for determining a concentration of a vitamin D component comprising:

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a releasing composition; and

a detecting composition,

10 the releasing composition comprises an aqueous base component and facilitates in releasing the vitamin D component from a vitamin D component binding-protein, the detecting composition facilitates in determining the concentration of the vitamin D component.

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2. A kit of claim 1 being useful for determining the concentration of the vitamin D component present in a mammal fluid.

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3. A kit of claim 1 wherein the mammal fluid is selected from the group consisting of milk, whole blood, serum, plasma and mixtures thereof.

LA<sup>B</sup>

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4. A kit of claim 1 wherein the mammal fluid comprises a human serum.

LA<sup>B</sup>

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5. A kit of claim 1 wherein the vitamin D component is selected from the group consisting of a metabolite of vitamin D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub>, D<sub>5</sub>, and D<sub>6</sub>.

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6. A kit of claim 1 wherein the vitamin D component comprises a 25-OH-D.

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7. A kit of claim 1 wherein the vitamin D component comprises a 1, 25-(OH)<sub>2</sub>-D.

8. A kit of claim 1 wherein the aqueous base component comprises NaOH.

9. A kit of claim 1 wherein the aqueous base component 5 comprises KOH.

10. A kit of claim 1 wherein the releasing composition comprises about 0.1 to about 1.0 M of the aqueous base component.

11. A kit of claim 1 wherein the releasing composition comprises about 0.35 to about 0.5 M of the aqueous base component, wherein the aqueous base component is NaOH.

15 12. A kit of claim 1 wherein the releasing composition is substantially free of an organic solvent.

13. A kit of claim 1 wherein the releasing composition further comprises a cyclo-oligomer component.

20 14. A kit of claim 13 wherein the cyclo-oligomer component comprises a cyclodextrin.

25 15. A kit of claim 13 wherein the cyclo-oligomer component is selected from the group consisting of alpha-cyclodextrin and beta-randomly methylated cyclodextrin.

30 16. A kit of claim 13 wherein the releasing composition comprises about 0.01 to about 5% of the cyclo-oligomer component.

35 17. A kit of claim 13 wherein the releasing component comprises about 2% of the cyclo-oligomer component, wherein the cyclo-oligomer component is an alpha-cyclodextrin.

18. A kit of claim 13 wherein the releasing component comprises about 0.05% of the cyclo-oligomer component, wherein the cyclo-oligomer component is a beta-randomly methylated cyclodextrin.

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19. A kit of claim 1 wherein the releasing component further comprises about 0.5 to about 5% of a metal salicylate, including sodium salicylate.

10 20. A kit of claim 1 wherein the releasing component further comprises about 0.01 to about 0.1% of a surfactant.

15 21. A kit of claim 20 wherein the surfactant is selected from the group consisting of tween-20 and triton X-100.

22. A kit of claim 1 wherein the releasing composition forms a homogeneous mixture with a mammal fluid.

20 23. A kit of claim 1 wherein the releasing composition comprises

about 0.1 to about 1.0 M of an aqueous base component;

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about 0.01 to about 5% of a cyclo-oligomer component; and

about 0.01 to about 5% of a metal salicylate.

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24. A kit of claim 23 wherein the aqueous base component is NaOH, the cyclo-oligomer component is cyclodextrin and the metal salicylate is sodium salicylate.

35 25. A kit of claim 1 wherein the detecting composition comprises a host component and a partner component,

wherein the host component binds to the partner component to form a partner/host complex, the concentration of the complex is proportional to the concentration of the vitamin D component.

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26. A kit of claim 25 wherein the host component comprises an antibody, portions thereof, or mixtures thereof.

10 27. A kit of claim 25 wherein the host component is labeled with a chemiluminescent label, a fluorescent label or a radio-active label.

15 28. A kit of claim 25 wherein the host component is an antibody labeled with acridinium.

20 29. A kit of claim 25 wherein the partner component comprises a vitamin D component linked to a separator component, the separator component is a solid phase or an antibody.

30. A kit of claim 29 wherein the separator component comprises a magnetic particle.

25 31. A kit of claim 29 wherein the partner component comprises a vitamin D component linked to a magnetic particle.

30 32. A kit of claim 31 wherein the partner component competes with the vitamin D component to bind to the host component.

33. A kit of claim 32 wherein the host component is an antibody labeled with acridinium.

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34. A kit of claim 32 wherein the partner component

binds to the host component through at least one intermediate binding component.

35. A kit of claim 34 wherein the intermediate component  
5 is labeled.

36. A kit of claim 34 wherein the intermediate component  
is labeled and the host component is not labeled.

10 37. A kit of claim 34 wherein at least one intermediate  
binding component comprise a vitamin D binding-protein.

15 38. A kit of claim 25 wherein the partner component  
competes with a vitamin D component to form a complex  
with the host component, the partner component comprises  
a vitamin D component linked to a magnetic particle, the  
partner component binds to the host component through a  
vitamin D binding-protein, the host component comprises  
an antibody labeled with acridinium.

20 39. A kit of claim 38 wherein the concentration of the  
complex is inversely proportional to the concentration of  
the vitamin D component.

25 40. A kit of claim 1 wherein the concentration of the  
vitamin D component is determined with a higher precision  
than that of an assay kit relying on an organic solvent,  
to release the vitamin D component from the binding-  
protein.

30 41. A kit of claim 1 wherein the releasing composition  
forms a homogeneous mixture with a body fluid containing  
the vitamin D component.

35 42. A kit for determining a concentration of a vitamin D  
component comprising:

a releasing composition comprising about 0.1 to about 1.0 M of NaOH or KOH, 0 to about 5% of a cyclodextrin, 0 to about 5% of salicylate and 0 to about 5 0.1% of a surfactant,

a detecting composition comprising an antibody labeled with acridinium and a partner component, wherein the partner component competes with a vitamin D component 10 to form a complex with the antibody, the partner component comprises a vitamin D component linked to a magnetic particle, the partner component binds to the antibody through a vitamin D binding-protein.

15 43. A kit of claim 42 wherein the releasing composition comprises about 0.35 to about 0.5 M of NaOH, about 2% of alpha-cyclodextrin and about 2% of salicylate, the releasing composition being substantially free of an organic solvent.

20 44. ~~A releasing composition comprising an aqueous base component, wherein the releasing composition facilitates in releasing a vitamin D component from a binding-protein, the releasing composition being useful in determining the concentration of a vitamin D component.~~

25 45. ~~A releasing composition of claim 44 wherein the aqueous base component comprises NaOH.~~

30 46. ~~A releasing composition of claim 44 wherein the aqueous base component comprises KOH.~~

47. A releasing composition of claim 44 comprising about 0.1 to about 1.0 M of the aqueous base component.

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48. A releasing composition of claim 44 comprising about

0.35 to about 0.5 M of the aqueous base component, wherein the aqueous base component is NaOH.

49. A releasing composition of claim 44 being substantially free of an organic solvent.

50. A releasing composition of claim 44 further comprising a cyclo-oligomer component.

10 51. A releasing composition of claim 50 wherein the cyclo-oligomer component comprises a cyclodextrin.

15 52. A releasing composition of claim 50 wherein the cyclo-oligomer component is selected from the group consisting of alpha-cyclodextrin and beta-randomly methylated cyclodextrin.

20 53. A releasing composition of claim 50 comprising about 0.01 to about 5% of the cyclo-oligomer component.

25 54. A releasing composition of claim 50 comprising about 2% of the cyclo-oligomer component, wherein the cyclo-oligomer component is an alpha-cyclodextrin.

25 55. A releasing composition of claim 50 comprising about 0.05% of the cyclo-oligomer component, wherein the cyclo-oligomer component is a beta-randomly methylated cyclodextrin.

30 56. A releasing composition of claim 44 further comprising about 0.5 to about 5% of a metal salicylate, including sodium salicylate.

35 57. A releasing composition of claim 44 further comprising about 0.01 to about 0.1% of a surfactant.

58. A releasing composition of claim 57 wherein the surfactant is selected from the group consisting of tween-20 and triton X-100.

5 59. A releasing composition of claim 44 wherein the releasing composition forms a homogeneous mixture with a body fluid sample containing the vitamin D component.

10 60. A releasing composition of claim 59 wherein a detecting composition is added to the homogeneous mixture to determine the concentration of the vitamin D component.

15 61. A releasing composition of claim 44 being employed in a biochemical assay.

62. A releasing composition of claim 61 wherein the biochemical assay is a homogeneous biochemical assay.

20 63. A releasing composition comprising

about 0.1 to about 1.0 M of an aqueous base component;

25 about 0.01 to about 5% of a cyclo-oligomer component; and

about 0.01 to about 5% of a metal salicylate, the releasing composition is useful in determining the concentration of a vitamin D component.

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64. A releasing component of claim 63 wherein the aqueous base component is NaOH, the cyclo-oligomer component is cyclodextrin and the metal salicylate is sodium salicylate.

65. A releasing component of claim 63 being substantially free of an organic solvent.

66. A method of assaying a body fluid sample for the concentration of a vitamin D component, the method comprising the steps of:

releasing the vitamin D component from the vitamin D component binding-protein by contacting the sample with a releasing composition in a holder; and

determining the concentration of the vitamin D component.

67. A method of claim 66 wherein the vitamin D is released into a homogeneous mixture of the body fluid sample and the releasing composition.

68. A method of claim 66 wherein the releasing composition comprises

about 0.1 to about 1.0 M of an aqueous base component;

0 to about 5% of a cyclo-oligomer component;

0 to about 5% of a metal salicylate; and

0 to about 0.1% of a surfactant.

69. A method of claim 68 wherein the aqueous base component is NaOH, the cyclo-oligomer component is cyclodextrin, the metal salicylate is sodium salicylate and the surfactant is tween-20.

70. A method of claim 66 wherein the releasing

composition is substantially free of an organic solvent, including an organic solvent.

71. A method of claim 67 wherein the determining step 5 includes the steps of:

10 adding a detecting composition to the holder, the detecting composition comprises a host component and a partner component, the host component binds to the partner component to form a partner/host complex;

isolate the complex in the tube;

15 measuring the concentration of the complex by measuring the concentration of the host component in the complex, the concentration of the complex is proportional to the concentration of the vitamin D component.

20 72. A method of claim 71 wherein the host component is an antibody labeled with acridinium.

25 73. A method of claim 72 wherein the concentration of the host is measured by detecting the emitted level of chemiluminescence.

30 74. A method of claim 71 wherein the partner component competes with a vitamin D component to form a complex with the host component, the partner component comprises a vitamin D component linked to a magnetic particle, the partner component binds to the host component through a vitamin D binding-protein.

35 75. A method of claim 66 wherein the determining step includes the steps of:

adding a partner component and a vitamin D binding-

protein to the tube, the partner component competes with the vitamin D component to bind to the vitamin D component binding-protein to form a partner/binding-protein complex;

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isolate the partner/binding-protein complex in the tube; and

10 adding a host component, the host component binds to the partner/binding-protein complex to form a partner/binding-protein/host component complex.

15 measuring the concentration of the partner/binding-protein/host component complex by measuring the concentration of the host component in the complex, the concentration of the complex is proportional to the concentration of the vitamin D component.

20 76. A method of claim 75 wherein the host component is an antibody labeled with acridinium.

25 77. A method of claim 75 wherein the concentration of the host is measured by detecting the emitted level of chemiluminescence.

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78. A method of claim 75 wherein the partner component comprises a vitamin D component linked to a magnetic particle.

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79. A method of claim 66 providing a more precise determination of the vitamin D component as compared to method using a releasing composition comprising an organic solvent.

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80. A method of assaying a body fluid sample for the concentration of a 25-OH-D component, the method

comprising the steps of:

releasing the 25-OH-D from the 25-OH-D binding-protein by contacting the sample with a releasing composition in a holder, including a cuvette, the releasing composition comprises about 0.1 to about 1.0 M of an aqueous base component, 0 to about 5% of a cyclo-oligomer component, 0 to about 5% of a metal salicylate, and 0 to about 0.1% of a surfactant; and

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adding a detecting composition to the holder,

the detecting composition comprises an antibody labeled with acridinium, a 25-OH-D binding-protein and a partner component, the partner component comprises a 25-OH-D linked to a magnetic particle,

the partner component competes with the released 25-OH-D to bind to the 25-OH-D binding-protein to form a partner/binding-protein complex,

the antibody binds to the partner/binding-protein complex to form an antibody/binding-protein/partner component complex;

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isolating the antibody/binding-protein/partner component complex in the tube; and

measuring the concentration of the antibody/binding-protein/partner component complex by measuring the concentration of the labeled antibody in the complex, the concentration of the complex is proportional to the concentration of the vitamin D component.

35 81. A method of claim 80 wherein the concentration of the labeled antibody is measured by detecting the emitted

level of chemiluminescence.

82. A method of claim 80 wherein the releasing composition comprises about 0.35 to about 0.5 M of NaOH, 5 about 2% of alpha-cyclodextrin and about 2% of salicylate.

83. A method of claim 80 wherein the releasing composition is substantially free of an organic solvent, 10 including an organic solvent.

84. A method of assaying a body fluid sample for the concentration of a vitamin D component, the method comprising the steps of:

15 forming a homogeneous mixture of a body fluid sample containing the vitamin D component and a releasing component; and

20 adding a detecting composition to the mixture to determine the concentration of the vitamin D component.